

RESEARCH ARTICLE

## Morphological study of the gastrointestinal tract of the snow trout, *Schizothorax esocinus* (Actinopterygii: Cypriniformes)

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**ABSTRACT.** The present study aimed to investigate the macroscopic structure of the gastrointestinal tract (GIT) of *Schizothorax esocinus* Heckel, 1838. The surface architecture of the buccopharynx, oesophagus and the entire intestinal tract of *S. esocinus* has been examined under scanning electron microscope (SEM) after fixing in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.3 for 18–48 hours and post-fixation for two hours at room temperature in 1% osmium tetra oxide buffered at pH 7.3 with 0.1 M cacodylate. The mucosal surface of buccopharynx, esophagus, intestinal bulb, and intestine reveal prominent longitudinal major or primary mucosal folds which are further subdivided into the series of irregular and well-circumscribed folds called minor or secondary folds. However, in the intestinal bulb and intestine, the longitudinal major or primary folds themselves form wavy or zigzagging patterns along the mucosal surface. The fine structure of the surface epithelium further shows that the apical surfaces of the epithelial cells are ped with finger-print like microridges, arranged in various patterns and regularly spaced. The rectal mucosa, on the other hand, displays a highly irregular type of major mucosal folds. The separation can't be seen between major mucosal folds. A thin film of mucous spread over the mucosal folds and the numerous pores through which mucous cells release their content has also been noted along the rectal mucosa. This investigation suggests the possible role of different digestive organs in relation to feeding, digestion, storage, absorption, and various other physiological processes, thereby providing a knowledge necessary to the understanding of pathological or physiological alterations in both aquaculture and natural environment.

**KEY WORDS.** Buccopharynx, esophagus, intestinal tract.

### INTRODUCTION

Ultrastructural studies are important to understand the relationship between physiological and biochemical functions and molecular mechanisms of an animal (Kalhor et al. 2018). The morphological studies of the gastrointestinal tract (GIT) of teleost fish received attention and have shown a significant diversity of morphological and functional characters (Kapoor et al. 1976, Fange and Grove 1979). Moreover, due to several morphological variations among the GIT of many fish species, nutritional requirements have been the focus of research in the field of fish ecology. Scanning electron microscope (SEM) has been used to describe the alimentary canal of teleosts morphologically to provide a detailed information on the structure and function of various anatomical structures in relation to their

different feeding adaptations (Grau et al. 1992, Yashpal et al. 2006). There are several studies on the morpho-physiological characteristics of the GIT of fish (Murray et al. 1994, Park and Kim 2001). Carrassón and Matallanas (1994) used SEM to study the digestive tract in *Dentex dentex* (Linnaeus, 1758); while the same technique was used by Mir and Channa (2010) to examine the intestinal tract of *Schizothorax curvifrons* Heckel, 1838.

The snow trout, *Schizothorax esocinus* Heckel, 1838 (Cypriniformes: Cyprinidae), is a native freshwater teleost fish that inhabit cold streams, rivers, and lakes (Menon 1999). In the Jammu and Kashmir state of India, it is mainly found in the inland waters viz. Mansbal lake, Dal lake, Lidder stream, river Jhelum (Kullander et al. 1999), The population of *Schizothorax* Heckel, 1838 have declined drastically in their natural habitats due to many environmental factors like habitat destruction,

eutrophication, climate change and chemical pollution (Mir and Channa 2009). Snow trout are highly preferred food fishes among local people because of its nutritional value and taste that fetches high market price (Sing and Paul 2010). Details of the digestive tract of this benthopelagic species is of vital physiological significance to better understanding of the features of this tract in relation to its function as it is the site of temporary storage of food besides digestive and absorptive process. *Schizothorax esocinus* lacks true stomach, as observed in other cyprinids, the proximal portion of the intestine is of greater diameter than the rest of the intestine and acts as a temporary storage organ for the ingested food (Mir and Channa 2010). There is a dearth of information regarding the scanning electron microscopy of the digestive tract of fishes of Kashmir in general and *Schizothorax* in particular. The purpose of the present study is to investigate the detailed ultrastructure of mucosal modifications of the entire digestive tract of naturally feeding fish, *S. esocinus* by using SEM. The study will provide an insight in understanding the possible role of different digestive organs in relation to feeding, digestion, storage, absorption, defecation as well as in the physiological processes, acting as a control for investigations in relation to pathology, pollution and stress conditions in both natural and polluted water bodies besides its application in fish culture.

## MATERIAL AND METHODS

All procedures were carried out according to the ethical principles of animal experimentation adopted by the Ethics Committee on Animal Use of the University of Kashmir, India. Live specimens of snow trout, *S. esocinus*, weighing 300 to 350 g (total length ranging from 25 to 32 cm) were collected from different freshwater sources of Kashmir valley. After being anesthetized with tricaine methanesulfonate, MS 222, solution by using a dosage of 100 mg L<sup>-1</sup>, the fishes were euthanized via a longitudinal incision through their ventral side. After dissection, various segmental tissue fragments of the alimentary tract viz. buccopharynx, esophagus, intestinal bulb, intestine and rectum of 2 cm long were removed and immediately processed for scanning electron microscopic studies. The esophagus, intestinal bulb, intestine, and rectum were incised and split longitudinally to expose the mucosal surface and rinsed in normal saline to remove excess mucus and other adhered particles. The tissues were then fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.3 for 18–48 hours. Following this primary fixation, the samples were then thoroughly rinsed three times in 1 M cacodylate buffer and post-fixed for two hours at room temperature in 1% osmium tetroxide buffered at pH 7.3 with 0.1 M cacodylate (secondary fixation). The post-fixed tissues were washed three times in buffer, dehydrated in ascending grades of ethanol and transferred to a 100% amyl acetate bath. The tissues were then critical-point dried through liquid CO<sub>2</sub> in Hitachi HCP-2 dryer and mounted on aluminum stubs with silver paint. These were sputter-coated with gold in a Hitachi

HUS-5GB high vacuum evaporator. The coated specimens were examined in a Hitachi S-3000H scanning electron microscope.

## RESULTS

Scanning electron microscope observations of the mucosal surface of the buccopharynx, esophagus, intestinal bulb and intestine of snow trout, *S. esocinus* reveal prominent longitudinal mucosal folds or major mucosal folds which left deep furrows in between (Fig. 1). The apical surfaces of the stratified epithelial cells of buccopharynx were sculpted with a network of well-defined and prominent linear elevations called microridges as revealed by the fine structure of the surface epithelium (Figs 2, 3).

The primary longitudinal and secondary mucosal folds in the epithelium of the esophagus are visible along with goblet cells (Figs 4, 5). Though extensively branched and connected, the microridges were observed to be regularly spaced bearing shallow groove between them which help in holding the mucus. These irregular elevations were most prominent in the anterior portion of the esophagus (Fig. 5). The mucous secreting oval shaped goblet cells, extensively scattered mucin droplets and numerous pores, representing openings for mucous release, were also noted on the surface of the epithelial cells of mucosal folds (Fig. 5).

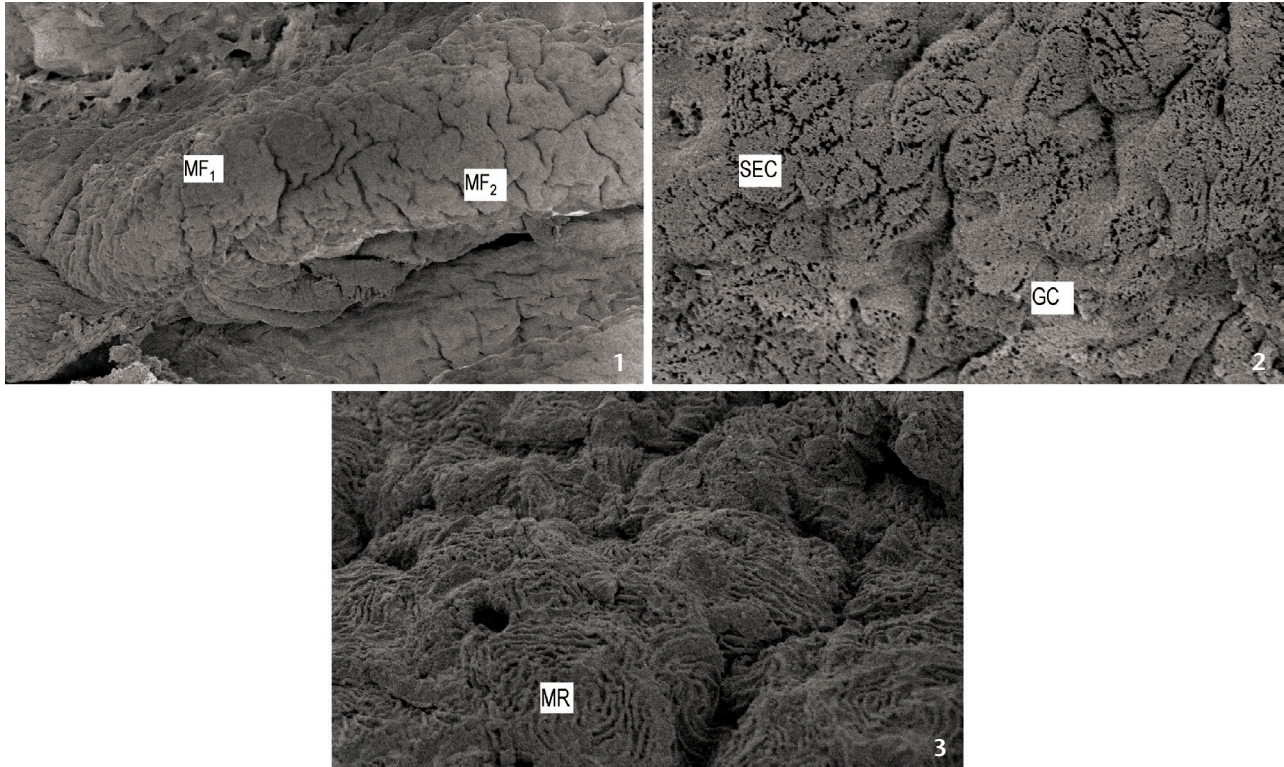
The SEM observation on the anterior intestine shows the organization of longitudinal mucosal folds (Fig. 6). A zigzagging or chevron-like pattern of arrangement of primary mucosal folds (major mucosal folds) which course along the long axis of the intestine bulb and intestine were also observed (Fig. 6). Moreover, the angles of the zigzag patterns were noted to be quite distant. The round-topped major mucosal folds of the intestinal bulb showing microridges were observed to be larger in size than the intestine (Figs 7, 8). The space between the primary longitudinal folds was large in the intestine bulb. These folds were lined with columnar cells bearing microvilli on their apical surfaces and appeared as a honeycomb polyhedral epithelial cells in the form of pentagonal or hexagonal elevations with the presence of gastric pits in between. When compared with the intestine bulb, the intestine has less distinct separation and the continuous cavity runs parallel throughout the entire length. Smaller infoldings of the mucosa (secondary folds) were noted to be present on the sides of the main primary folds (Fig. 9). The rectum, on the other hand, exhibits a highly irregular pattern of major mucosal folds. The secondary mucosal folds (minor mucosal folds) and well-defined cavities were not observed in the present study, however, irregular mucosal folds were seen (Fig. 10). The rectal mucosal folds were covered by a thin film of mucus and were beset with distinct pores through which the mucous cells discharge their contents.

## DISCUSSION

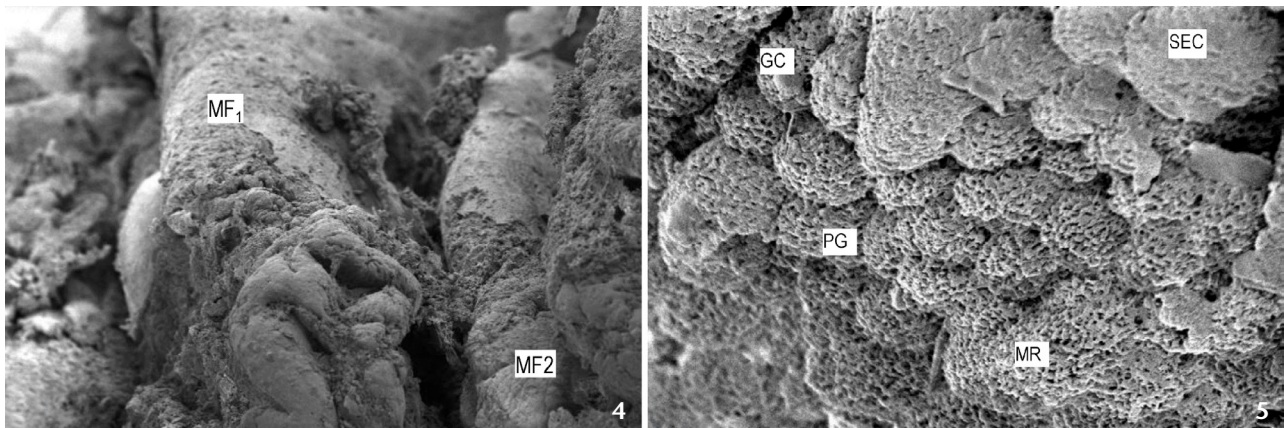
The morpho-anatomical features of the digestive tract of Indian freshwater teleosts, in relation to their feeding habits, are well documented (Sinha and Moitra 1975, Sinha and Chakrabarti

1984, 1985). According to these authors, densely coiled intestine, modification of mucosal folds and complexity of the intestinal villi are the main features associated with the herbivorous mode of feeding. It has been observed in the present study that the

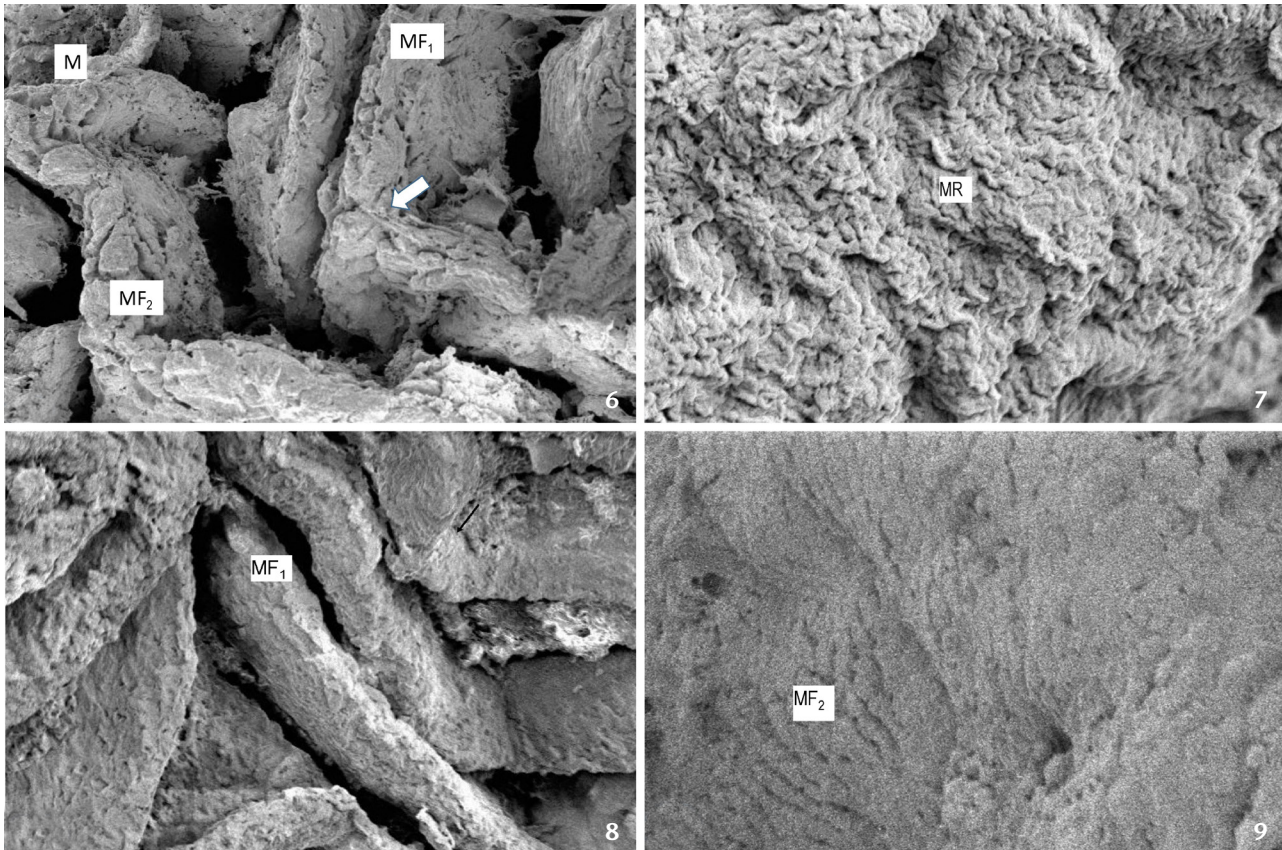
mucosal folds in the various regions of the alimentary tract of the fish studied get modified in different ways. The buccopharynx and esophagus display a well-elaborated pattern of primary or major folds, which are further subdivided into many secondary



Figures 1–3. Scanning electron micrograph of buccopharynx showing: (1) primary longitudinal (MF<sub>1</sub>) and secondary folds (MF<sub>2</sub>); (2) goblet cell (GC) and stratified epithelial cells (SEC); (3) microridges (MR). Scale bars: 1 = 200  $\mu$ m, 2 = 20  $\mu$ m, 3 = 10  $\mu$ m.



Figures 4–5. Scanning electron micrograph of esophagus showing: (4) primary longitudinal (MF<sub>1</sub>) and secondary mucosal folds (MF<sub>2</sub>); (5) stratified epithelial cells (SEC), goblet cells (GC), Pores of goblet cells (PG) and microridges (MR). Scale bar: 4 = 500  $\mu$ m, 5 = 10  $\mu$ m.



Figures 6–9. Scanning electron micrograph of intestinal bulb showing: (6) primary or major ( $MF_1$ ) and secondary mucosal folds ( $MF_2$ ), covered with thin film of Mucin (M). The zig-zag arrangement of primary folds is clearly visible in this region ( $\leftarrow$ ); (7) microridges (MR); (8) primary or major mucosal folds ( $MF_1$ ) and the zig-zag invagination ( $\leftarrow$ ); (9) secondary folds ( $MF_2$ ). Scale bar: 6 = 500  $\mu\text{m}$ , 7 = 10  $\mu\text{m}$ , 8 = 100  $\mu\text{m}$ , 9 = 20  $\mu\text{m}$ .

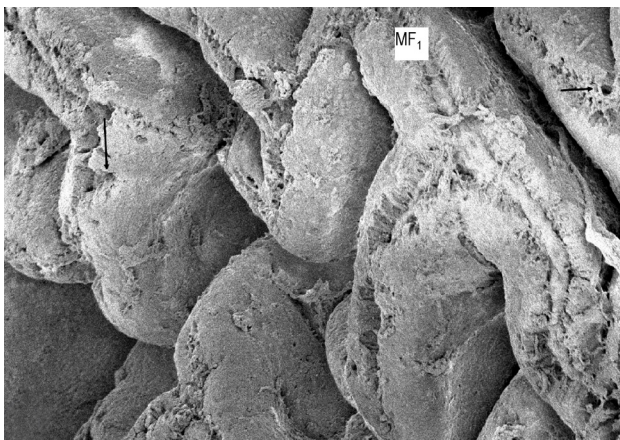


Figure 10. Scanning electron micrograph of rectum showing irregular mucosal folds ( $MF_1$ ), mucous ( $\downarrow$ ) and pores ( $\rightarrow$ ). Scale bar: 200  $\mu\text{m}$ .

These primary and secondary folds aid in swallowing. Similar mucosal foldings have been observed in carp, *Catla catla* (Hamilton, 1822) by Sinha and Chakrabarti (1985) and in *Serola dumerili* (Risso, 1810) by Grau et al. (1992). The apical surfaces of the stratified epithelial cells of buccopharynx and the luminal surface of the stratified epithelial cells of the esophagus are beset with a complex pattern of microridges, which increase the surface area and provide the channel for mucus transport. According to Harding (1973), the microbridge on the epithelial surfaces are subjected to mechanical insult. The presence of microridges may, therefore, represent a mechanical adaptation which in the buccopharynx and esophagus would withstand the trauma resulting from ingested materials. The microridges of the buccopharynx and esophagus by virtue of their nature seem to spread and hold mucous film secreted by the adjacent mucous cells over the soft mucous membrane. These observations are compatible with the studies made earlier (Ezeasor and Stokoe 1980, Sinha and Chakrabarti 1985, Murray et al. 1994).

The secretion of mucous serves as a lubricant in transmitting food from buccopharynx to esophagus thereby enabling the esophagus to act as a transit tube for ingested food from the oropharyngeal cavity to the intestinal bulb. The secretion of mucous by the mucous cells to the exterior has been reported by several authors in various teleosts (Khanna 1964, Martin and Blaber 1984, Humbert et al. 1984, Sinha and Chakrabarti 1985, 1986, Chakrabarti and Sinha 1987, Tibbets 1997, Park and Kim 2001, Podkowa and Goniakowska-Witalinska 2003). Some authors have reported taste buds in the oesophageal wall of many teleosts (Ezeasor and Stokoe 1980, Fishelson et al. 2004). Sis et al. (1979) demonstrated taste buds only in the cranial portion of the esophagus of the channel catfish, *Ictalurus punctatus* (Rafinesque, 1818). However, during the present study, no taste buds were observed in the esophagus. This is in correlation with the findings of Elbal and Angulleiro (1986) and Grau et al. (1992) in different teleosts.

Fine anatomical features of the mucosal surface of the intestinal tract in *S. esocinus* as revealed by present SEM study correlated with the other teleosts (Sinha and Chakrabarti 1985, 1986, Chakrabarti and Sinha 1987, Kapoor and Khanna 1994, El-Shammaa et al. 1995), it has been observed that the mucosal folds in various regions of the intestine get modified in different ways. The major mucosal folds noted to be very high, consist of zigzagging pattern that course along the long axis of the entire intestine and form deep cavities between them. The major mucosal folds along with the minor ones around the cavities not only serve to increase the total surface area but also retain ingested food for longer periods in the stomach less fish studied. The deep cavities in the intestinal region are an adaptational feature meant mainly for storage. It is well known that the primary function of this region is absorption and secretion. Nevertheless, partial retention of the semi-digested food also occurs while the same passes through the zigzag course of this region. The zigzagging pattern of the major mucosal folds has been reported by McVay and Kann (1940) and Caceci (1984). According to Curry (1939), the tops of the mucosal folds in the intestinal bulb are flat, round or pointed. However, during the present study, the tops of the mucosal folds in the entire intestine are flat and rounded. Caceci (1984) also observed flat and round folds in the intestinal mucosa of goldfish, *Carassius auratus* (Linnaeus, 1758). The luminal surface of the entire intestine in *S. esocinus* is lined with well-developed columnar epithelial cells. The luminal plasma membrane of the columnar epithelial cells of the anterior portion of the intestine sculpted into microridges. Sinha and Chakrabarti (1985) report that the microridges represent a mechanical adaptation in the intestine. According to various authors, the microridges are not only restricted to the intestinal surface but have been found to occur also on the epidermal cells of the body (Hawkes 1974, Lanzing and Higginbotham 1974, Harris and Hunt 1975, Hunter and Nayudu 1978, Bereiter-Hahn et al. 1979, Whitear 1990), epithelial cells of the gills (Mattey et al. 1980, Eiras-Stofella and Charvet-Almeida 1998) and the

stratified epithelial cells of buccopharynx and oesophagus (Mallatt 1979, Sis et al. 1979, Clarke and Witcomb 1980, Ezeasor and Stokoe 1980, Humbert et al. 1984, Sinha and Chakrabarti 1985, MacDonald 1987, Grau et al. 1992, Murray et al. 1994).

The rectum reveals irregular mucosal foldings that increase the surface area of the rectal mucosa. The rectum plays a very little role in food storage, digestion and absorption as evidenced by the low mucosal folds as well as the absence of well-defined cavities and the minor mucosal folds, thereby bearing a close relationship with its functional aspects. A thin film of mucous secretion has been observed in the rectal mucosa, which aids in easy defecation. The occurrence of few mucous cells in between the epithelial cells of the various regions of the digestive tract of various teleosts has been reported by different authors (Khanna 1964, 1968, Sinha 1975, Park and Kim 2001). According to the authors mentioned above, the presence of copious mucin discharged by the mucous cells in the different regions of the alimentary canal of various teleosts is an adaptation for adequate lubrication of food for facilitating conduction posteriorly along the alimentary canal.

On the basis of our results, the surface architecture of the mucosa of the digestive tract of *S. esocinus* throws light on the modifications of the mucosal epithelium and morphological characteristics of the various cells lining the alimentary canal which are useful in correlating the morphological features with function. These findings provide insights into the structure and functioning of the digestive system in this fish. Further detailed studies should be conducted on the ultrastructure level of the gastrointestinal tract of *S. esocinus*.

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